

## Overview

Understory macroalgal assemblages are important sources of primary production in marine environments. Studies of how interactions between canopy and understory species influence the productivity of the entire community are rare, particularly in marine systems where measuring primary production of diverse assemblages of macroalgae is logistically challenging. To overcome these challenges, we developed a simple model of primary production for understory macroalgae that relates species-specific light use relationships measured in the laboratory to biomass and light levels measured in nature (Miller et al. 2012). Here we describe the methods that we used to measure respiration, photosynthesis at non-saturating irradiance ( $\alpha$ ) and photosynthesis at saturating irradiance ( $P_{\max}$ ) of 22 species of understory macroalgae and juvenile *Macrocystis pyrifera*, which are common to kelp forests off Santa Barbara, CA.

## Laboratory-measured rates of photosynthesis

Twenty-three of the most common species of macroalgae were collected from reefs near Santa Barbara at a depth of 5–9 m and kept in an indoor aquarium with running seawater at ambient temperature for no longer than two days before photosynthesis versus irradiance (P vs. E) measurements were made. We used whole thalli in the incubation experiments to incorporate the effects of plant morphology and self-shading into production measurements. Each algal specimen was cleaned of all epiphytes prior to incubation. The holdfast and most of the stipe of the stipitate kelps *Pterygophora californica*, *Egregia menziesii*, and *Laminaria farlowii* were removed so the specimens could fit into the incubation tanks; most of the photosynthetic tissue in these species is in their blades. Juveniles approximately 30 cm in height were measured for the giant kelp, *Macrocystis pyrifera*.

Photosynthesis (P) versus Irradiance (E) relationships were obtained for each species by anchoring a specimen with modeling clay in a natural upright position to the bottom of a sealed acrylic tank (volume 35 L). The tank was submerged in a bath of running seawater. Tanks were equipped with a submersible aquarium pump (Rio model 50; 262 L h<sup>-1</sup>) to provide circulation, and an optical probe (D-Opto; ENVCO) that measured dissolved oxygen at a frequency of once per minute. Specimens were incubated in the dark for 20 minutes to measure respiration rate. Tank seawater was then sparged with nitrogen gas (N<sub>2</sub>) to lower initial oxygen concentrations. The nitrogen sparging had no detectable effects on seawater pH. Irradiance was provided by two 500-W halogen lamps fixed 30 cm above the tanks. Plastic mesh screens (9 in total) were sequentially removed from the incubation tank lid at 20-min intervals, creating incubation irradiances of 10 (all screens present), 19, 36, 60, 103, 178, 198, 344, 392, and 700  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , which spanned the range of irradiances measured in the field. The wet mass and volume of each specimen were measured following the completion of incubations. Wet samples were dried for at least 72 h at 60° C and re-weighed to obtain dry mass. Tank volumes were corrected for the volume displaced by algae, clay, pump, and oxygen probe. Oxygen evolution rates for each light level and for dark incubations were calculated by fitting a linear regression to the measured change in oxygen concentration over incubation time. The regression equation was used to calculate hourly rates of oxygen evolution per gram of dry photosynthetic tissue. Oxygen evolution rates were converted to carbon using a photosynthetic quotient of 1. The initial slope of

the curve,  $\alpha$ , was calculated by fitting a linear regression to the change in production rate over a range of non-saturating irradiance values ( $1\text{--}150 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) for each taxon (Jassby and Platt 1976).  $P_{\text{max}}$  was estimated individually for each taxon by fitting the data to the equation presented below using a least squares non-linear fitting procedure (SAS ver. 9.1.3, PROC-NLIN; SAS Institute, Cary, NC).

We used the methods of Miller et al. (2012) to measure photosynthesis, irradiance, and respiration by 23 of the most common macroalgal taxa observed at long-term study sites surveyed by the SBC LTER. Additionally, we measured P versus E for the reproductive fronds of *Cystoseira osmundaceae*, which can exhibit seasonally high biomass. We incubated whole thalli (minus the woody stipe and holdfast in the case of the kelp *Pterygophora californica*) in clear acrylic tanks and measured oxygen evolution at nine levels of irradiance (19, 36, 60, 103, 178, 198, 344, 392, and  $700 \mu\text{mol m}^{-2} \text{sec}^{-1}$ ;  $n = 10$  to  $20$  whole thalli per taxon) that encompassed the 95<sup>th</sup> percentile of daylight values recorded on the bottom at our sites during the period of study. The initial slope of the relationship between photosynthesis and irradiance at non-saturating irradiance ( $\alpha$ ) was determined using linear regression of non-saturating irradiance values for each taxon (Jassby and Platt 1976). Photosynthesis at saturating irradiance ( $P_{\text{max}}$ ) was estimated for each thallus by fitting the hyperbolic tangent function (Jassby and Platt 1976) using SAS (SAS Institute Inc., North Carolina version 9.1.3). Estimates of  $P_{\text{max}}$  and  $\alpha$  were averaged across replicate thalli to obtain mean estimates for each species or taxonomic group. Units of oxygen were converted to carbon using a photosynthetic quotient of 1.0 (following Rosenberg et al. 1995) and respiration and production rates were standardized to the dry mass of photosynthetic tissue.

## References

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